MICROBIOLOGY OF FLAX RETTING

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Linum usitatissimum has been cultivated for thousands of years as a textile fiber producing plant. The Egyptians must have raised it, since their mummies are found today wrapped in fine linen. Frequent allusions to flax and linen in the Bible indicate that the ancients were acquainted with the usefulness of the bast fibers in flax and had methods of separating them from the rest of the plant. They were also familiar with other types of fibers, since these are found in their papyri today. The United States cannot be regarded as a great flax or linen producing country; it has had to depend mainly on importation to supply the increasing demands for linen. In the spinning of flax the United States was at the bottom of the list of the larger countries in 1915, with slightly over 8000 spindles against Great Britain's 1,161,000.[1] Most of these were in Ireland, although they were not kept busy on fiber produced in Ireland. Russia was once the largest flax fiber producing country, contributing 80 per cent of the flax fiber used in making linen. Since the world war, however, this has changed on account of the industrial disorganization in that country. Statements in the press, said to come from the Office of Fiber Investigation of the United States Department of Agriculture, indicate that the spinning mills in this country have used about 10,000 pounds of flax fiber per annum. For the production of this amount of fiber about 60,000 acres of land would be required. In 1920 only 6000 acres of flax were grown in this country, while the low price paid for it will restrict the acreage to about 3000 in the future.

Flax is raised in this country mostly for seed which is pressed for linseed oil; a smaller amount is raised for the fiber. Flax raised for seed is of a different quality from that usually required for fiber. Fiber flax is taller and produces less seed. It requires greater care in cultivation, and especially careful handling at the

[1] MILES, C. F., Fiber flax. U.S. Dept. Agric. Farmer's Bull. no. 669. 1915.

harvest. Some claim that it must be pulled, not cut, and tied up carefully in bundles. This may be one reason why it has been difficult to utilize the flax from seed flax for spinning. It might be possible in the future to combine profitably the seed and fiber crop. This would tend to reduce the value of each crop taken by itself perhaps, but the value of the combined crops of seed and fiber might compensate for any decrease in the value of the single crop.

The bast fibers, which are those used in making linen, are cemented to the other parts of the stalk and to each other by means of materials, for convenience, called pectins. Undoubtedly this term is used only in a general way to cover a number of compounds closely related chemically. The aim of the retting process is to remove these "binders" without harming the cellulose fiber. The fermentation must be checked when these fibers have been freed by the hydrolysis of the pectose or salts of pectic acid. These binding materials which hold the stalk together are undoubtedly carbohydrate in nature, and thus susceptible to the action of microoganisms.

Preparation of flax fiber

The fiber is prepared from the flax straw by a special process which seems to have been built up after a long period of time without much assistance from the sciences. Proper harvesting is very important. Fiber flax should be pulled either by hand or by machinery and tied into bundles which are shocked for curing. Cutting the flax is claimed by some to leave the ends of the stalk exposed for undesirable decompositions. When the heads are shocked for curing, this cut end becomes susceptible to the attacks of undesirable bacteria. The fibers become badly stained also. This may not be entirely true, however, under actual practice.

After curing, the stalks are retted. This is really a rotting process, which indicates the origin of our present term. Three general methods may be used to dissolve the binder which holds the cellulose fibers to the woody materials: dew retting, water retting, and chemical retting. The first two only are of bacteriological interest, and were studied in the present investigation.

Dew retting was used by our forefathers in this country for preparing flax fiber for spinning. It represents the earliest method of preparing flax fiber. No special apparatus is needed, since the flax straw is merely spread on the ground in the fall and allowed to remain throughout the winter. Dew retting has been used for the preparation of most Russian flax fiber. Its greatest objection is the time required, but this may be reduced greatly by carrying the process out under conditions where the retting organisms may be made to work harder.

Water retting was introduced undoubtedly to get away from certain of the distinct disadvantages of dew retting. It is carried out either in slow flowing rivers or in ponds and other inclosed bodies of water. The bundles of flax straw are packed into these basins and weighted down. The retting process starts with a gaseous fermentation of the carbohydrate materials in the flax straw. If conditions are favorable, a little over ten days is necessary for the completion of the fermentation. The flax should be removed when all the pectic materials are dissolved, or over-retting will result. The bundles are removed, dried in sun and air, and are then ready for scutching. The river Lys in Belgium is famous for its flax retting. River retting has certain economic features which limit its wide application. As KUHNERT[2] has shown, the stream becomes putrescible, which is detrimental to fish life. It carries amounts of organic materials in the reduced conditions which may give off objectionable odors. Water retting has not had wide application in this country.

Several attempts have been made to improve on the water retting. One of the earliest of these was proposed by SCHENCK in 1846. The flax straw was packed tightly into a tank and the water kept at a temperature of 75°–95° F. This warm environment was more favorable to the development of the bacteria concerned in this process, and a vigorous fermentation quickly established itself. The vats had all of the characteristics of a fermentation mixture. Others have proposed similar methods with a higher temperature.

Scutching is the process by which the woody material is broken away from the cellulose fibers after they have been retted and

[2] KUHNERT, Landw. Wochenbl. Schleswig-Holstein **70**:540–543. 1920.

dried. Different methods have been used, all of which rest on breaking the woody particles and mechanically removing them from the stalk. The fibers are finally combed to separate the "tow" from the fibers which are not long enough to remain in line. The latter may be used in paper, coarse linen, etc. The fiber from flax may be 30–40 inches in length, thus yielding a product which is valuable for spinning.

Microbiology of retting

Retting is indeed a natural process, and may be regarded as merely a step in the cycles of the elements. The various factors involved have been separated in an attempt to intensify certain ones in order to make the process shorter, and also to produce a better fiber. In retting flax man has simply made use of and intensified a reaction which is always going on.

One of the first investigations on the microbiology of retting was carried out by VAN TIEGHEM[3] in 1879, in his study of the process of water retting. An anaerobic organism named *Bacillus amylobacter* was reported as the organism which quickly decomposed the pectic materials of the flax stalk. In the same year VAN TIEGHEM stated that his *Bacillus amylobacter* was probably identical with the *Vibrion butyrique.* An aerobic spore-forming organism was also found by WINOGRADSKY.[4] FRIBES, working in this laboratory, tried various disinfectants for sterilizing the flax, but finally used the method of heating in water at 100° C. for three successive days, or at 115° C. for fifteen minutes. Various aerobes and anaerobes were isolated, none of which seemed to have any effects on the flax. Finally a specific anaerobe was isolated. It was a spore-former, the young cells of which were 10μ to 15μ by 0.8μ. Glucose, sucrose, starch, and lactose were fermented if some nitrogenous matter was present. A quite similar microorganism was also isolated by BEHRENS[5] from the water retting of hemp. The organism was a *Clostridium* form fermenting the binding materials of hemp straw.

[3] VAN TIEGHEM, Sur la fermentation de la cellulose. Bull. Soc. Bot. France **26**:25. 1879.

[4] WINOGRADSKY, S., Sur le rouissage du lin et son agent Microbien. Comp. Rend. Acad. Sci. **121**:742. 1895.

[5] BEHRENS, J., Cent. Bakt. **10**:524. 1903.

It fermented glucose, sucrose, fructose, lactose, galactose, and starch with abundant gas formation, but could not attack arabinose, cellulose, gum arabic, or calcium lactate. Similar to WINOGRADSKY'S organism, it required some source of nitrogen, as peptones or proteins. It was an obligate anaerobe with large spores which had a greater diameter than the vegetative rod.

In 1902 HAUMANN[6] published an interesting paper which gave an entirely new aspect to the subject. He stated that many common microorganisms could ret flax. He first studied the flora on the stalks of retted flax and isolated a number of organisms, among which were *Bacterium coli-communis*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Streptothrix Forsteri*, *Penicillum glaucum*, *Cladosporium herbarum*, *Bacillus mesentericus fuscus*, *B. mycoides*, *B. termo*, *Micrococcus roseus*, and *Mucor mucedo*. The mere presence of these organisms would not indicate that they functioned in retting. The preponderance of certain species, however, might indicate some relation to the retting process. *Cladosporium herbarum*, *Bacillus mesentericus*, *B. subtilis*, and colonies of *Streptothrix* were common. To determine whether these organisms were important, HAUMANN inoculated sterile flax with pure cultures. The flax stalks were put into long culture tubes plugged with cotton. The tubes containing flax were heated to temperatures below 110° C. in the dry condition. He stated that three heatings under such conditions did not alter the flax. Retting was accomplished by using many of the common species of microorganisms. There was a difference in action, *Pseudomonas fluorescens* giving good results, while *Micrococcus roseus* was least satisfactory. HAUMANN concluded from this that all of the common bacteria were able to ret flax. Some of these bacteria were also able to split pectin. His results are unique in that they contradict those secured by many others and also those obtained in this investigation. In the light of some of the recent work on thermal resistance of the spores of anaerobic bacilli, HAUMANN'S method of sterilizing the flax is open to criticism. One would not expect these spores to be destroyed by a temperature of 110°, especially in the dry conditions. It is

[6] HAUMANN, M. L., Étude microbiologique et chimique du rouissage aerobic du lin. Annals Past. Inst. **16**:378–385. 1902.

possible that some of the spores of anaerobic bacteria survived and produced the characteristic change in the flax fiber which was attributed to the pure culture of aerobes used.

An anaerobic organism was also found by BEIJERINCK and VAN DELDEN,[7] to which they gave the name *Granulobacter pectinovorum*. This organism was an obligate anaerobe and a vigorous spore-former. It required protein or its split products as sources of nitrogen, liquefied gelatin, and actively attacked carbohydrates. In general it had the characteristics of the anaerobes described by others, especially the one found by WINOGRADSKY.

A more recent extensive investigation has been reported by STORMER,[8] who found an anaerobic spore-former which he called *Plectridium pectinovorum*. The granular structure of the cells makes one believe that he had the organism described by earlier writers. STORMER'S bacillus seemed to differ from these, however, in being a facultative anaerobic organism.

In sharp contrast with these papers are a number of others indicating that an aerobic organism is involved. MARMIER[9] mentioned such an organism. BEIJERINCK and VAN DELDEN also reported that *Bacillus subtilis* and *B. mesentericus* would ret flax, although they found another organism, which they called *Granulobacter pectinovorum*, which seemed to ret flax more completely and quickly.

ROSSI[10] studied the retting of vegetable fibers and stated that the microbiological retting process has certain advantages over chemical retting. Whether this is true or not is probably determined by the uses to which the fiber will be put. He devised an aerobic method in which *Bacillus comesii* was allowed to act on material which had been steeped in water at from 28° to 35° C. The pure culture was added and the vat was aerated, the tempera-

[7] BEIJERINCK, M. W., and VAN DELDEN, A., Over de bacterien welke bij roten van vlas werksdam zign. Kon. Ak. Wetensch. Amsterdam (Verstag. van de Gewone Vergadering der Wis en Natunkk) **12**:673. 1903.

[8] STORMER, K., Über die Wasserroste des Flachses. Chem. Zent. **76**:41. 1905.

[9] MARMIER, Le rouissage du lin. Bot. Centrabl. **83**:90. 1900.

[10] ROSSI, G., Industrial retting of textile plants by microbiological action. Bull. Agric. Intell. **8**:1067–1074. 1916.

ture being maintained between the extremes just mentioned, since the fermentation goes on most vigorously at that range. ROSSI's work was concerned with hemp, but the organisms may be similar to those used with flax. An anonymous article in the *Bulletin Imperial Institute* (17:605–607. 1919) confirms the work of ROSSI. Flax straw is immersed in water at 82° to 86° F. in vats, and after the addition of a special aerobic organism, *B. comesii*, it is aerated during the retting process. The pectinous materials are consumed and the retting process is completed in 36–40 hours. Another aerobic organism called *Bacillus felsineus* is reported which will also ret hemp, flax, ramie, nettle, and other plants. It is said to produce a rapid retting and furnish a fine, white, well separated fiber. LOESER,[11] in carrying out ROSSI's process, boiled flax for forty minutes and then treated it with pure cultures of *Bacillus comesii*. The vats were aerated and maintained at a temperature of 30°–32° C. These methods are more expensive than those which do not require boiling. Furthermore, the anaerobic organisms are so resistant to heat that they might pass through these preheating procedures and function in retting the flax, although the credit be given to certain aerobic bacteria.

CORRIGAN[12] has more recently discussed the relation of fungi to retting. His statements apparently are based on the researches which have been reviewed herein. Mrs. WYANT[13] has recently reported briefly some results on this problem. She isolated about forty cultures of both aerobes and anaerobes. Each of these cultures was tested for its retting ability. This narrowed the work down to one pure culture which received more intensive study.

Experimental work

The work which has been carried out at this laboratory has been done with pure cultures, and on materials from a large rettery

[11] LOESER, R., Retting of flax by means of bacteria. Jour. Soc. Chem. Ind. 38:407. 1919.

[12] CORRIGAN, J. FREDERICK, Bacteria and molds: their biological nature and their influence on vegetable fibers. Jour. Soc. Dyers Colourists 36:198–201. 1920.

[13] WYANT, Z. N., Some bacteriological problems involved in the retting of flax. Abstracts Bact. 5:208, 209. 1921.

in eastern Michigan.[14] Most of the flax used in these experiments was grown in Michigan, and was thoroughly cured before it was received at the laboratory. It was tied in bundles or "heads" and was in fine condition, since it had been cultivated for fiber. The other was the flax which had been raised for seed, and consequently had not been kept tied in bundles, but had become badly broken and bent during thrashing. While just as good retting was secured on this as with the stalks which were raised for the fiber and tied in bundles, the resulting fiber was not of good quality. Perhaps such a raw material could be better retted by the chemical process. The fibers were much shorter, but probably just as satisfactory for paper making.

The samples from the retting vats were taken in sterile bottles and subjected to the usual bacteriological examination. Both aerobic and anaerobic plates were made, from which pure cultures were picked and transplanted into various culture media. The flora from the vats was varied, but spore-forming bacteria of both aerobic and anaerobic types were common. The aerobic types were similar to the members of the *subtilis-mesentericus* group; they formed large spreading colonies on solid media and liquefied gelatin very rapidly. Spores were easily formed in large numbers.

The pure culture experiments were carried out with many of the common bacteria and several cultures of yeasts. The bacteria and yeasts used were:

Pseudomonas pyocyaneus	Saccharomyces cerevisiae
Proteus vulgaris	Torula monosa
Erythrobacterium prodigiosus	Saccharomyces ellipsoideus
Pseudomonas fluorescens	Saccharomyces marxianus
Zopfii Zenkerii	Myoderma vini
Bacterium enteritidis	Bacterium cloacae
Bacillus gasoformans	Bacillus cereus
Bacterium colon	Bacterium capsulatum
Bacillus butyricus	Erythrobacillus arborescens
Bacterium aerogenes	Bacillus subtilis

[14] The author is indebted to Mr. B. S. SUMMERS of the Summers Linen Company of Port Huron, Michigan, for material upon which some of the results here reported were secured, and for his constant interest in the progress of the work. Dr. R. E. FINDFUSZ of the American Writing Paper Company kindly furnished some flax straw and exhibited a kindly interest.

Contrary to the statements of HAUMANN, successful retting could not be accomplished with these common bacteria. None of the organisms such as *Bacillus subtilis* was found to possess any activity. In order to test the retting ability of all these cultures and those isolated from vat liquors, flax straw was cut into pieces about 4.5 inches long. These were put into long test tubes and covered with water. Sterilization was accomplished by heating in the autoclave at 115° C. for fifteen minutes. The sterility of these flax straw culture tubes was determined by both aerobic and anaerobic cultures in sterile litmus milk and other media. Heating in the autoclave at 115° C. seemed to be sufficient for their sterilization, and did not seem to injure the straw or make the retting more quickly accomplished when the bacteria were applied. Experiments were carried out later in 3 gallon earthenware jars in order to test the retting activity of the organism which was finally isolated. Attempts were also made to find an aerobic organism such as was used by ROSSI. Flax straw was put into a large glass vat covered with distilled water and aerated for a week, with frequent examinations for aerobic pectin fermenting organisms.[15] No success was obtained, even after specimens of soil and decaying organic matter were added. Several workers have mentioned the use of aerobic strains. One who is familiar with bacterial metabolism would expect to find an anaerobic organism. Members of this group decompose more materials than aerobes which leave so much energy in their products. The anaerobes partially hydrolyze large amounts of material for a certain amount of energy, while the aerobes hydrolyze a smaller amount of material completely for the same amount of energy. Perhaps for the same reason the anaerobes are used in most of the fermentations which yield certain organic chemicals.

The study of the vat liquors from a large commercial rettery in eastern Michigan, and from the specimens of flax and retting liquors in small tubs in the laboratory, narrowed down to an anaerobic organism as the most specific. This was secured in pure cultures by anaerobic plating in plain agar. Transfers made into other media allowed the following characterization:

[15] Industrial retting of textile plants by microbiological action. Bull. Agric. Intell. 8:1067–1074.

Vegetative cells: The vegetative cells grown on common media were large rods with a dense protoplasm. Many of the cells presented a granular structure. Iodine staining indicated the presence of starchy materials.

Spores.—Spores are formed which are larger than the vegetative cells, giving the cells the shape of a *Clostridium*. The spores were found to resist heating for thirty minutes at 80° C. Further studies on their thermal resistance seemed uncalled for in a study of this nature.

FERMENTATION REACTIONS.—Large amounts of gas were formed in lactose, glucose, saccharose, and glycerol. In most of these fermentation tubes there was a pronounced odor of butyric acid.

LITMUS MILK.—Litmus milk was quickly decomposed; the curd was peptonized with large amounts of organic acids, principally butyric.

GELATIN.—Gelatin was quickly liquefied at 20° C.

PLAIN BROTH.—The broth was rendered cloudy with a precipitate only after a long period of growth.

NITRATES.—Nitrates were reduced with the formation of nitrites and ammonia.

That this anaerobic organism is common in nature and on the stalks of the flax plant was shown in several ways. It was found to be present repeatedly on the stalk of flax by simply soaking it in distilled water. After about thirty hours a vigorous evolution of gas would start, which ceased in about forty-eight hours at room temperature. This could be reproduced at will. That the organism is present in soil was shown by adding garden soil to tubes of sterile flax in distilled water. All evidences of a rapid retting started in twenty-five hours, and was completed in forty-eight hours. Pure cultures of this anaerobe removed the carbohydrate binders in the flax stalk in forty-eight hours at room temperature (26°–32° C.). The fermentation is accompanied by a vigorous evolution of gas, which is forced out of the stalk, clinging to the side until the bubble is large or some jar removes it. The liquid becomes turbid and has a strong characteristic odor.

Whether pure cultures of this anaerobe would be valuable in retting is probably doubtful, since the organism is so widely distributed in nature. Several experiments were carried out to deter-

mine whether the organisms were on the flax stalk itself. There was no difficulty in the majority of attempts to demonstrate its presence. In a large sense the change brought about in the flax stalk is a natural one, which is continually going on in nature. It is an attempt on the part of nature to bring about the transformation of organic compounds, and to keep the elements moving through their cycles. In retting it is the desire to carry this to the point when the binding materials in the fiber are dissolved, thus releasing the bast fibers, and to check it just before the cellulose of the fibers is attacked. It is reasonable to expect that this could be done more quickly in a rettery, where favorable conditions are maintained and where the flora of microorganisms may easily be established.

The quality of the water seems to have great influence on the quality of fiber. During a few experiments in the beginning of this work tap water containing about 1 p.p.m. of iron was used. This yielded a fiber which was dark and discolored in appearance. The use of pure distilled water corrected this and yielded a silken glossy fiber nearly white in color. This supports the experience in water retting that a better fiber is secured where a softer water is available, and confirms the statements of workers that the quality of fiber produced in the Courtrai region in Belgium, where flax is retted in the waters of the river Lys, is superior to that retted elsewhere.

One of the earlier investigators stated that the presence of aerobic bacteria tended to produce more favorable conditions in which the anaerobic could act. To determine whether there was foundation for this, several experiments were carried out with mixtures of the anaerobe isolated in this investigation and certain common aerobes. *Bacillus subtilis* and *Bacterium coli* were used, but it could not be seen that they were of any value. Neither did they seem to lengthen or shorten the time required for completion of the retting process. Their presence seemed to have little effect. Under natural conditions they might favor the retting in that they would help to remove the products formed from the pectic binding materials. In favor of this assumption is the fact that nature does most of her work with mixed cultures, and many significant changes are brought about by symbioses.

Summary and conclusions

1. Retting of flax in the preparation of linen fiber is a natural process, and an attempt on nature's part to keep the elements moving through their cycles.

2. The organism isolated as the specific one in retting flax was *Clostridium amylobacter*. It is an anaerobic spore-forming bacterium which quickly hydrolyzes the carbohydrate "binders" in the flax stalk. It was found to be commonly present on flax stalks and widely distributed in nature.

3. Symbiosis of this organism with common aerobic bacteria did not seem to decrease the time required for retting or produce conditions under which the anaerobic *Clostridium amylobacter* could work better.

4. Temperature is an important factor in that it retards or increases the activity of the fermentation involved in retting. The best temperature seemed to be 30° C.

5. The retting process can be shortened and a better quality of fiber produced by carrying it out under controlled conditions where the optimum environment may be maintained.

6. Previous sterilization of the flax did not seem to affect the retting process. The flax retted as quickly when put into the water without previous treatment as when it was boiled or heated in the autoclave.

7. No real success was secured by the use of fifteen common aerobic bacteria and five yeastlike fungi.

8. Flax raised for seed was quickly retted, although the fiber was not in as good condition as that prepared from flax raised for fiber.

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